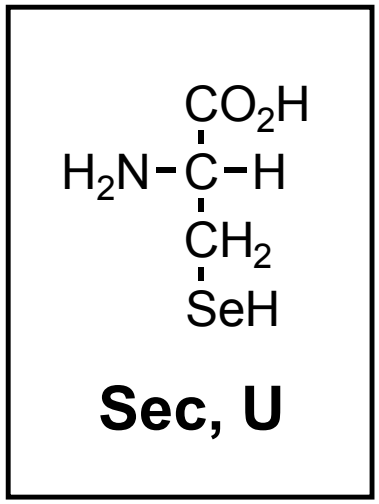
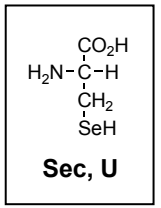
Selenocysteine: The 21st Amino Acid



Amanda L. Garner

October 5, 2007

Selenocysteine - Discovery



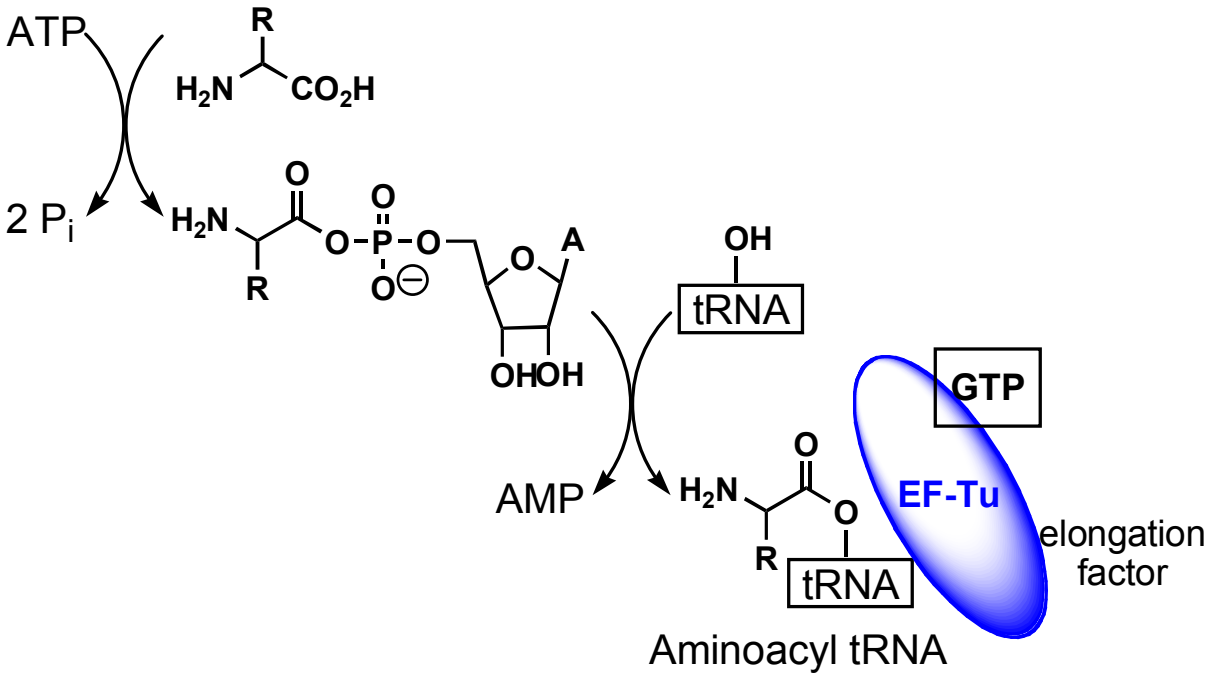
Chronology:

- Selenium (Se) initially believed to be toxic (more reactive than sulfur)
- 1954: First report of Se requirement for function of bacterial formate dehydrogenase
- 1959: First paper addressing selenocysteine (Sec)
- 1972: First biochemical studies on role of Se at enzyme level
- 1986: Key study implicating UGA as codon for Sec

Se currently regarded as essential nutrient in humans and mammals (development, immune function, male reproduction, aging process)

Pinsent, J. *Biochem. J.* **1954**, *57*, 10. Shum, A. C.; Murphy, J. C. *J. Bacteriol.* **1972**, *110*, 447. Bock, A. *PNAS* **1986**, *83*, 4650.

Translational Chain Elongation - Other 20 Amino Acids



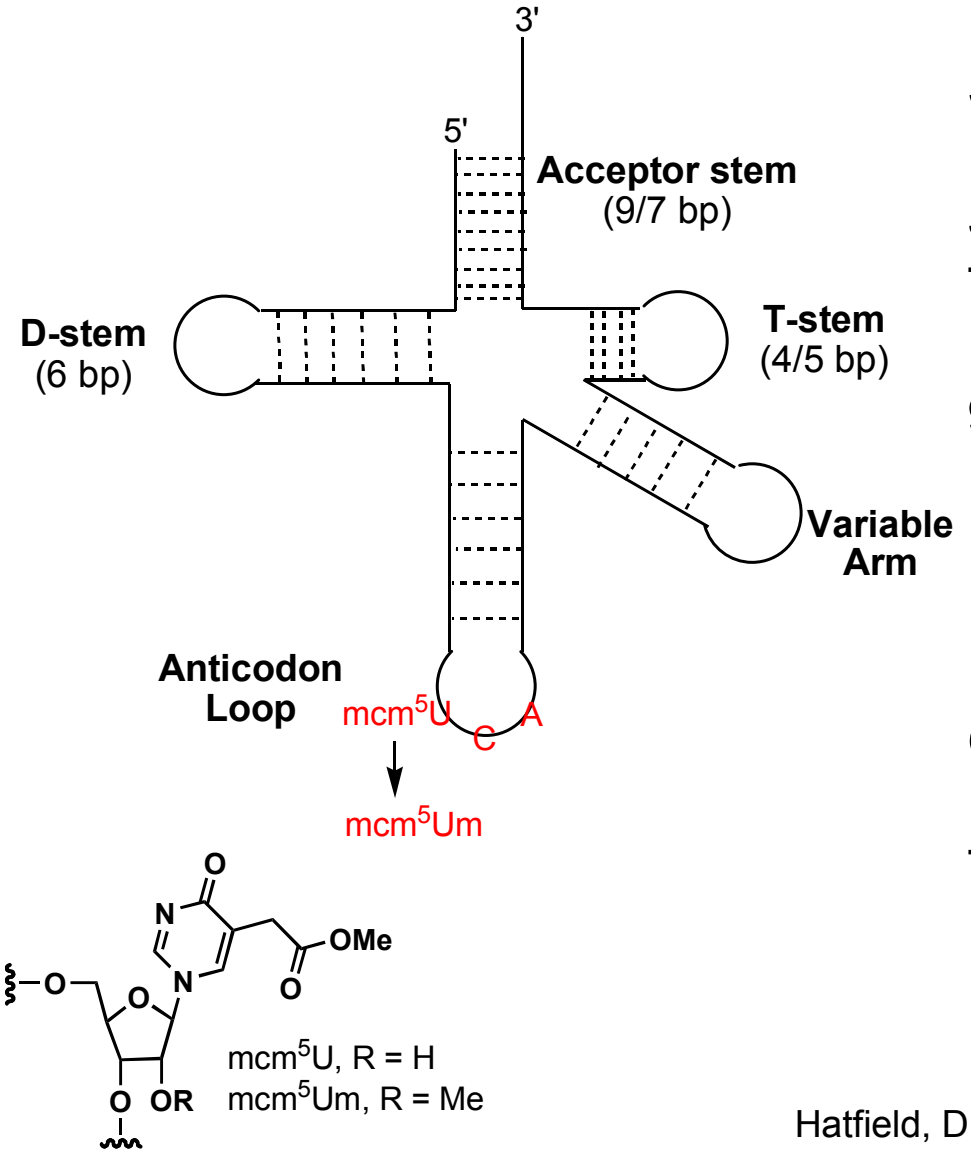
Molecular Biology of the Cell

Requirements for Sec Insertion into Protein

1. Sec $tRNA^{[Ser]Sec}$
2. UGA Codon
3. Selenocysteine Insertion Sequence (SECIS) elements
4. SECIS-binding protein 2 (SBP2)
5. Sec-specific elongation factor (EFsec)

Hatfield, D. L.; Gladyshev, V. N. *Mol. Cell. Biol.* **2002**, *22*, 3565.

Sec tRNA[Ser]Sec



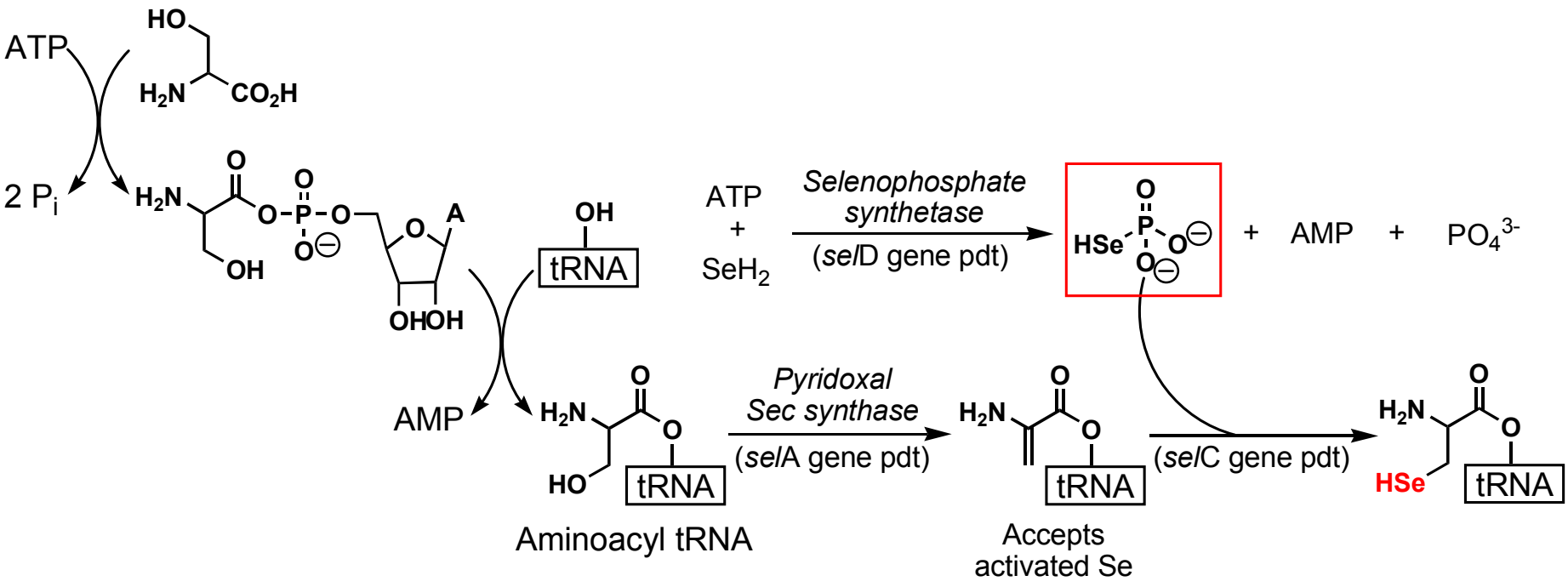
- Sec tRNA only known tRNA that governs entire class of proteins
- *sel*C gene product
- Two known structures: 9/4 and 7/5 cloverleaf
- 90 nt long (longest tRNA known)
  - 1 extra nt in acceptor and T-stems; 2 extra nt in D-stem; longest variable arm
- Highly conserved vs. other tRNAs
- Occurs as single gene copy in all mammals
- Two isoforms: mcm^5U or mcm^5Um (final step of Sec tRNA maturation; responsive to Se)

Hatfield, D. L.; Gladyshev, V. N. *Mol. Cell. Biol.* **2002**, *22*, 3565.

Sec tRNA$^{[Ser]Sec}$

First aminoacylated with Serine (Ser, S)

Mechanism well known in bacteria but not in eukaryotes



Hatfield, D. L.; Gladyshev, V. N. *Mol. Cell. Biol.* **2002**, *22*, 3565.

UGA Codon

UGA = termination codon and Sec codon

Discovered by Böck in 1986 in bacterial formate dehydrogenase (FDH)

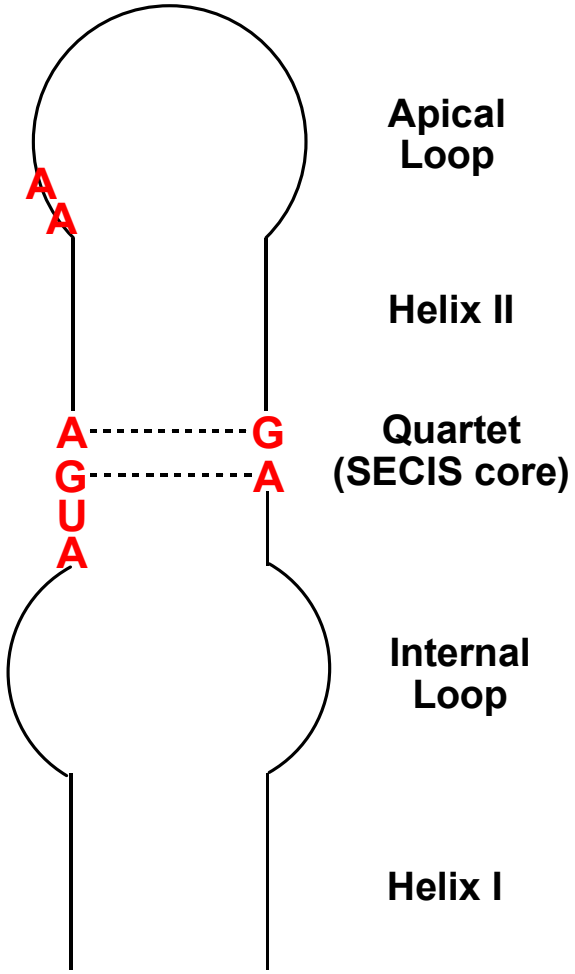
In vivo studies confirmed that Sec tRNA$^{[Ser]Sec}$ recognizes the UGA codon in both bacteria and eukaryotes (definitive that Sec is 21st amino acid)

What determines whether UGA will code for termination or Sec?

Bock, A. *PNAS*, **1986**, *83*, 4650. Bock, A. *J. Biol. Chem.* **1989**, *264*, 9720.
Hatfield, D. L. *J. Biol. Chem.* **1989**, *264*, 9724.

SECIS Elements

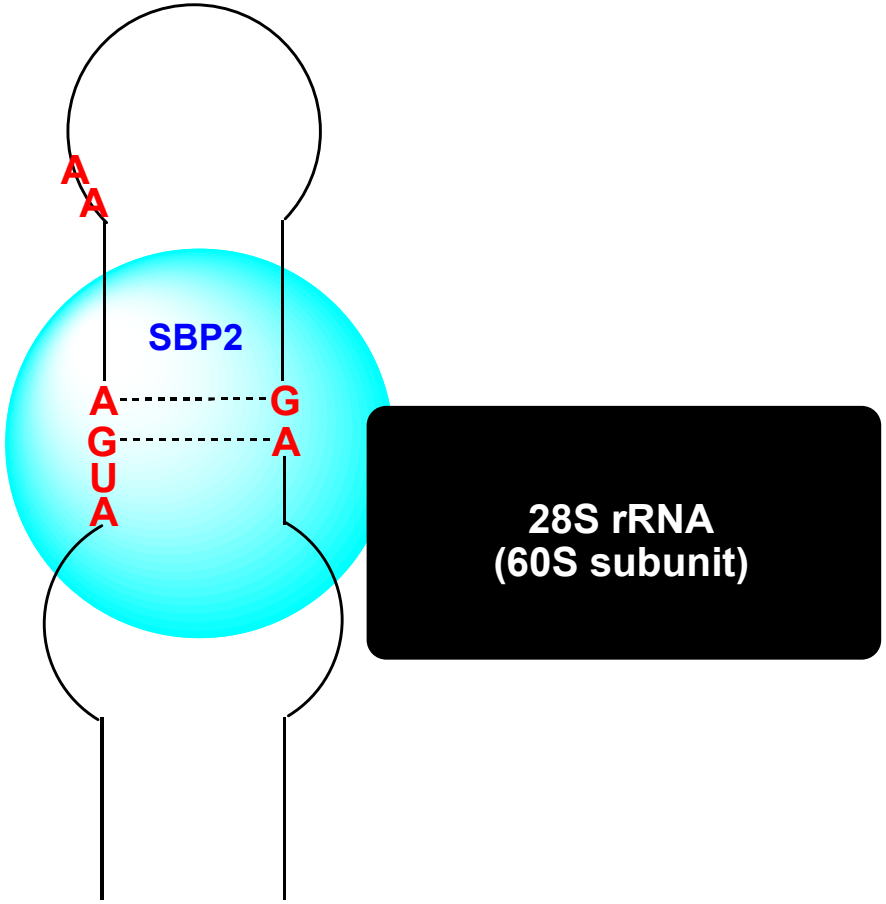
- Bacteria - found as 40 base sequence downstream from UGA
- Eukaryotes - found in 3'-UTR
- Primary sequence not conserved (except bases in red)
- Main functional site is non-Watson-Crick quartet
- Responsible for Sec insertion if can access ribosome



Hatfield, D. L.; Gladyshev, V. N. *Mol. Cell. Biol.* **2002**, *22*, 3565.

SECIS Binding Protein 2 (SBP2)

- Forms strong complex with SECIS quartet and sequence preceding (not apical loop)
- Stably associated with 28S rRNA of ribosome (preselects for Sec insertion)
- Also binds Efsec to recruit Sec tRNA$^{[Ser]Sec}$
- mcm^{5}Um may be involved in initiating complexation



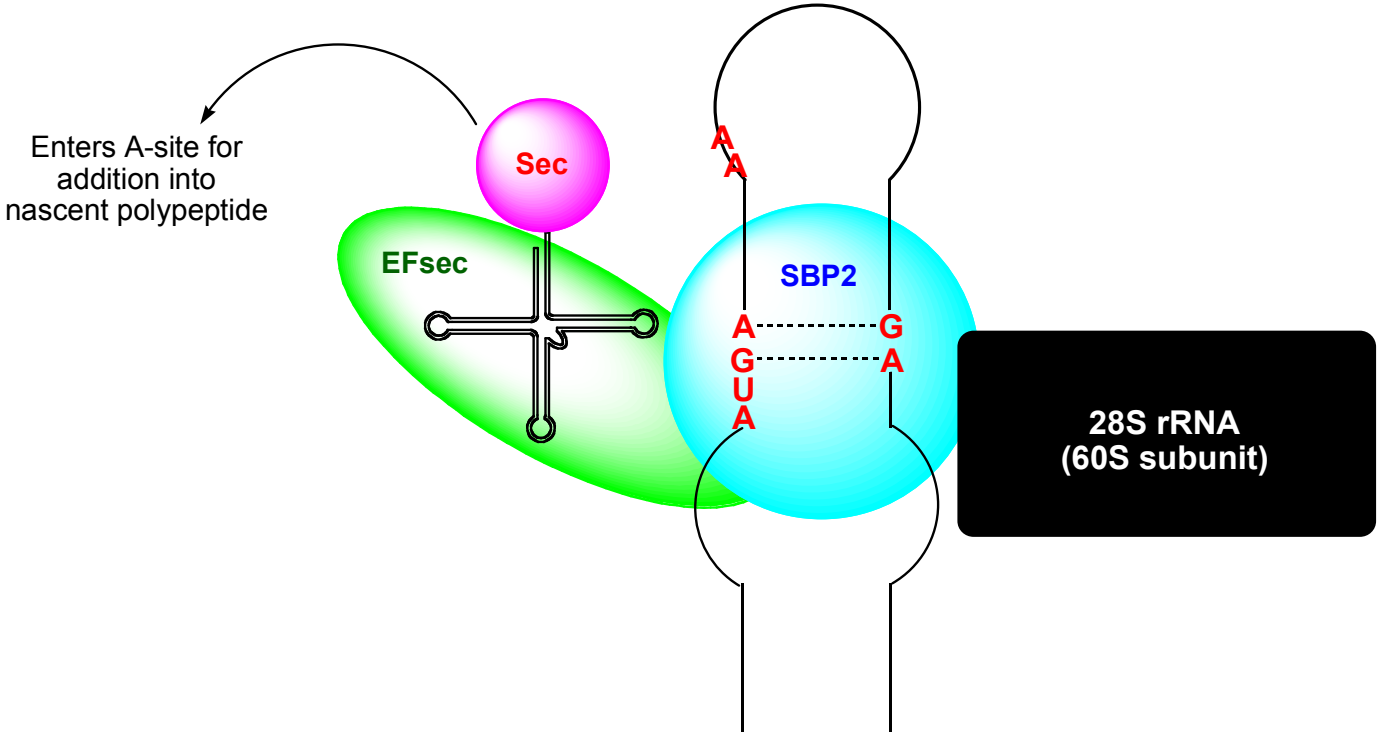
Hatfield, D. L.; Gladyshev, V. N. *Mol. Cell. Biol.* **2002**, *22*, 3565.

Elongation Factor EFsec

*sel*B gene product

Recognizes extra bp in acceptor stem of Sec tRNA$^{[Ser]Sec}$

Ready to insert Sec into protein in response to UGA codon



Hatfield, D. L.; Gladyshev, V. N. *Mol. Cell. Biol.* **2002**, *22*, 3565.

Selenocysteine - Evolution

Evolution - believed to be a latter addition to the existing genetic code of 20 amino acids

Sec found in bacteria, archaea, and eukaryotes

Bacteria/archaea - found in proteins responsible for catabolic processes

Eukaryotes - found in proteins responsible for anabolic and regulatory pathways

Increased use of Sec in proteins of higher vertebrates

*Sec is an evolutionary advancement

Gladyshev, V. N. *BioFactors*, **2001**, *14*, 87.

Examples of Known Selenoproteins

Prokaryotes:

1. Glycine reductase (first known selenoprotein; 1973)
2. Formate dehydrogenase
3. Selenophosphate synthetase

Eukaryotes:

1. Glutathione peroxidase (first known selenoprotein; 1973)
2. Thioredoxin reductase
3. Thyroid hormone deiodinase
4. Selenophosphate synthetase
5. Selenoprotein P

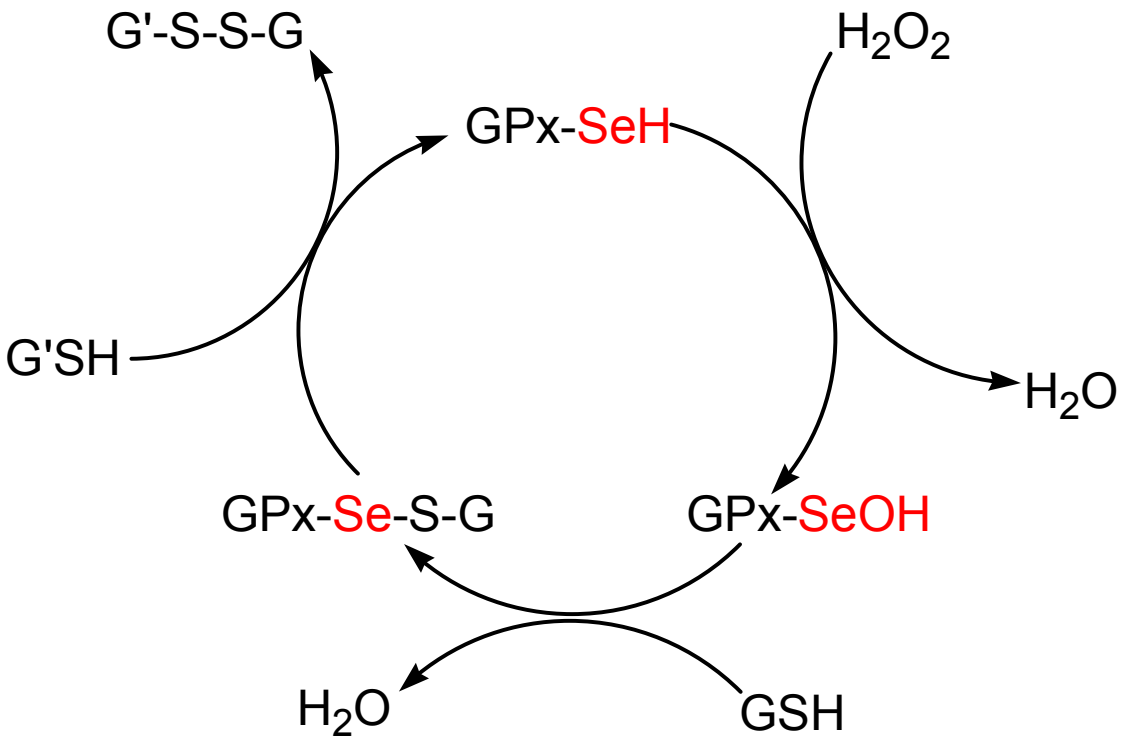
Only 12 eukaryote selenoproteins with known function

25 total selenoproteins contained in the human genome

Gladyshev, V. N. *BioFactors*, **2001**, *14*, 87. Burk, R. E., Hill, K. E. *Annu. Rev. Nutr.* **2005**, *25*, 215.

Glutathione Peroxidase

Key enzyme in oxidative stress by scavenging peroxides



GPx = glutathione peroxidase
GSH = glutathione

Schock, H. H. *FEBS Lett.* **1973**, *32*, 132. Sies, H. *Angew. Chem. Int. Ed.* **2003**,*42*, 4742.

Glutathione Peroxidase Structure

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

From human plasma

RCSB Protein Data Bank

Thioredoxin/Thioredoxin Reductase

Thioredoxin (Trx)

Major cellular protein disulfide reductase

Numerous important roles in mammals:

1. DNA synthesis (binds T7 DNA polymerase)
2. Redox regulation of transcription factors
3. Regulation of apoptosis (bind apoptosis signaling kinase)
4. Immunomodulation
5. Pregnancy and Birth
6. CNS

Contains important Trx fold (5 β-strands and 4 α-helices) and Cys-Gly-Pro-Cys active site sequence

Holmgren, A. *Eur. J. Biochem.* **2000**, *267*, 6102.

Thioredoxin Structure

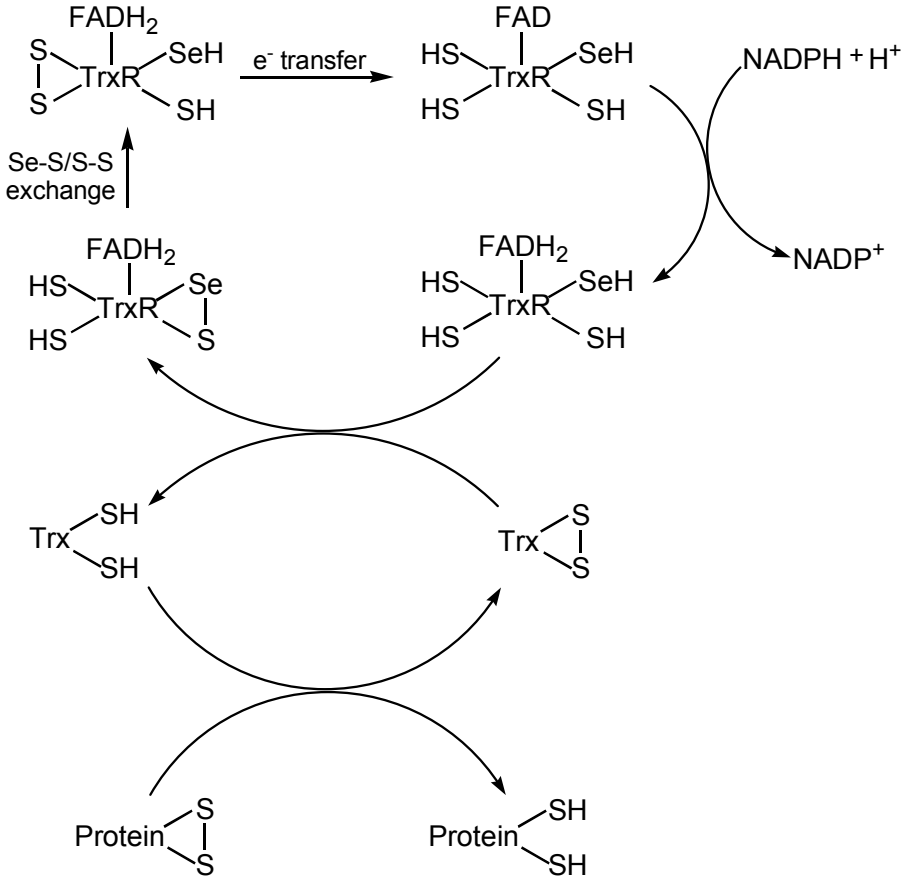
QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

RCSB Protein Data Bank

Thioredoxin/Thioredoxin Reductase

Thioredoxin reductase (TrxR; 1996, Stadtman)

Se essential for activity; Gly-Cys-Sec-Gly motif conserved



Redox potential of each couple unknown

Holmgren, A. *PNAS* **2000**, *97*, 5854. Sies, H. *Angew. Chem. Int. Ed.* **2003**, *42*, 4742.

Thioredoxin Reductase Structure

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

RCSB Protein Data Bank

Selenoprotein P

Abundant extracellular protein rich in Sec

Identified in 1977 in rat plasma; 1982 shown to contain Sec

Only expressed in vertebrates

First known protein with >1 Sec (10 total in human)

Two Sec-containing domains:

1. *N*-terminal domain - UxxC redox motif
2. *C*-terminal domain - 9 U and 10 C (no redox motif)

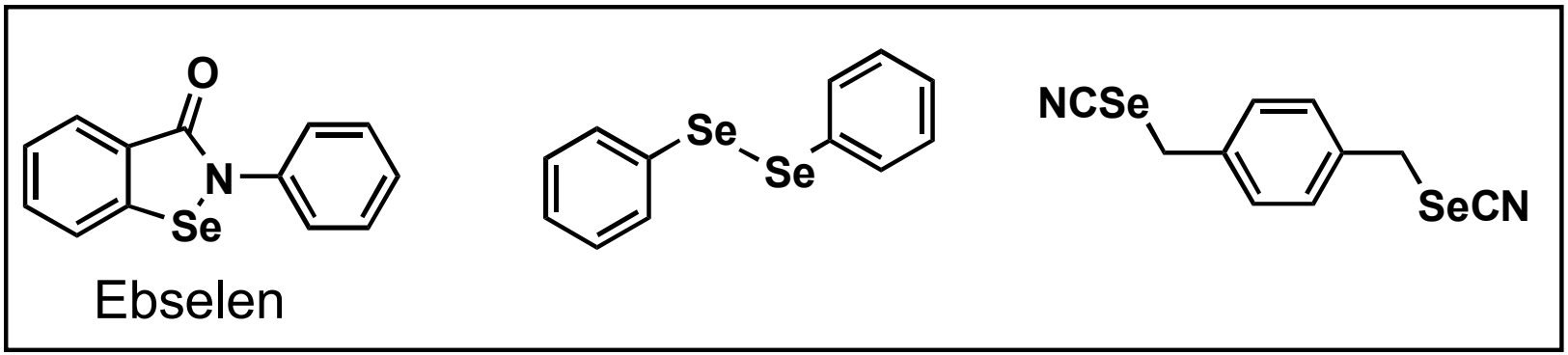
3D structure unknown but contains 3 Se-S motifs and 2 S-S motifs; 2 SECIS elements

Exhibits weak GPx activity but function unknown

Speculated as Se transport protein but no redox activity

Burk, R. F.; Hill, K. E. *Annu. Rev. Nutr.* **2005**, *25*, 215.

Organoselenium Pharmacology



Ebselen: GPx mimetic agent (anti-oxidant), anti-inflammatory activity, neuroprotection

Ph_2Se_2: GPx mimic, anti-inflammatory activity, ani-nociceptive activity (greater than Ebselen)

Selenocyanate: Chemopreventative (colon, breast, lung, liver, intestine, oral tissue carcinomas)

Noguiera, C. W. *Chem. Rev.* **2004**, *104*, 6255. Zeni, G. *Eur. J. Pharm.* **2007**, *555*, 129.

Uniqueness of Selenium

Cys and Sec least abundant aa in proteins

In adult human, 140 g S but only mg quantities of Se

Se more polarizable (better nucleophile, leaving group and lower redox potential)

RSeH: pKa = 5.7 vs. RSH: pKa = 8.5 (~1 pKa unit higher in protein)

RSeH largely deprotonated at physiological pH

Sies, H. *Angew. Chem. Int. Ed.* **2003**, *42*, 4742. Dawson, P. E. *J. Am. Chem. Soc.* **2006**, *128*, 16684.

Redox Properties of $RSeH/R_2Se_2$

$R\text{-}S\text{-}S\text{-}R + 2\,H^+ + 2\,e^- \longrightarrow 2\,RSH$ $\quad E^o = -233$ mV (-270 mV in Trx)

$R\text{-}Se\text{-}Se\text{-}R + 2\,H^+ + 2\,e^- \longrightarrow 2\,RSeH$ $\quad E^o =$ between -220 to -323 mV (also estimated as high as -488 mV)

- Only S nucleophile known to reduce Se-Se is dithiothreitol (DTT, -323 mV)
- GSH and cysteine also shown to reduce but at concentrations 10^3 greater than [Se-Se]
- Trx synthesized as double U mutant forming Se-Se
  - Redox properties substantially different from native protein (could not be reduced by β-mercaptoethanol)

Gunther, W. H. H. *J. Org. Chem.* **1967**, *32*, 3931. Dickson, R. C. *Arch. Biochem. Bioophys.* **1969**, *130*, 547. Bock, A. *Biochemistry* **1994**, *33*, 3404.

Cys→Sec Mutants

Mutation typically shows catalytic enhancement

Examples:

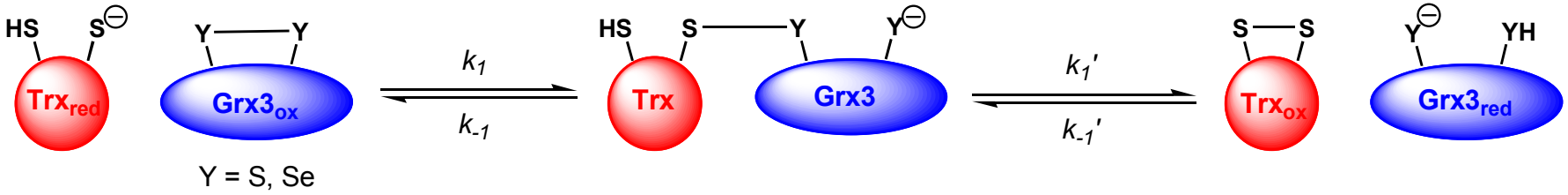
1. Formate dehydrogenase: k_{cat} = 9 s^{-1} (C); 2800 s^{-1} (U)
2. Deiodinase: C only 10-20% as active as U
3. GPx: C has insignificant activity

Although more active, little known about kinetic role of Se in enzyme active site

Implications for Sec as evolutionary enhancement

Stadtman, T. *Annu. Rev. Biochem.* **1996**, *65*, 83.

Synthetic Seleno-Glutaredoxin3 Analogues



Redox potentials:

- ^{11}Cxx^{14}C: -194 mV
- C11U: -260 mV
- C14U: -275 mV
- ^{11}Uxx^{14}U: -309 mV

Sec more active than Cys

(Reference: Trx = -270 mV; GSH = -240 mV)

~9000-fold enhancement for double mutant (relative to k_{-1})

Equilibrium studies show ~19% Se-Se reduced suggesting Se-Se could have role in biological catalysis if found in natural protein

Dawson, P. E. *J. Am. Chem. Soc.* **2006**, *128*, 16684.

Identification and Characterization of a Selenoprotein Family Containing a Diselenide Bond

Identified SelL, only known selenoprotein containing 2 Sec in UxxU redox motif

Only present in aquatic organisms:

- Eukaryotes - 11 fish, 2 ascidian, 2 crustacean, 1 mollusk
- Prokaryotes - 2 bacteria

Unknown why only found in aquatic organisms

Close Cys homolog found in mammals

Gladyshev, V. N. *PNAS* **2007**, *104*, 13919.

Identification and Characterization of a Selenoprotein Family Containing a Diselenide Bond

Structure:

- Prx-like domain in *C*-terminus
- Conserved UxxU in *N*-terminus
- 2 Sec in loop between β-strand and α-helix (Trx-like)
- Single SECIS element for 2 Sec (contains CA vs. AA as unpaired nt in apical loop)

SelL Structure: Figure 1A, 1B (2 Sec shown in red)

Gladyshev, V. N. *PNAS* **2007**, *104*, 13919.

Identification and Characterization of a Selenoprotein Family Containing a Diselenide Bond

1. Determine if single SECIS element can direct addition of 2 Sec

   Express 2 prokaryotic SelL sequences in *E. coli* via GST-fused fragment containing both UGA codons, SECIS element, and *C*-terminal His tag

   (Figure 2A, 2B, 2E)

Gladyshev, V. N. *PNAS* **2007**, *104*, 13919.

Identification and Characterization of a Selenoprotein Family Containing a Diselenide Bond

2. Confirmation of 2 Sec by mass spectrometry

   Method = LC-ESI MS/MS

   Mass (from neutral, +2, and +3) = 1039.3 with correct isotopic distribution

   (Figure 3A)

Gladyshev, V. N. *PNAS* **2007**, *104*, 13919.

Identification and Characterization of a Selenoprotein Family Containing a Diselenide Bond

3. Expression in eukaryote, *Danio rerio* (zebrafish)

   Constructs with *C*-terminal His-tagged SelL (and mutants ULPC, CLPU, CLPC)

   Transfected into HEK293 and NIH 3T3 cells

   (Figure 2C, 3C)

Gladyshev, V. N. *PNAS* **2007**, *104*, 13919.

Identification and Characterization of a Selenoprotein Family Containing a Diselenide Bond

4. Subcellular localization
   Expressed GST-fused *D. rerio* SelL in NIH 3T3 cells
   Cys mutant with GFP at terminus

   Observe cytosolic localization

   (Supporting Information)

Gladyshev, V. N. *PNAS* **2007**, *104*, 13919.

Identification and Characterization of a Selenoprotein Family Containing a Diselenide Bond

5. Redox properties of SelL
   Expressed in HEK293 cells (Cys→Ser)
   a. *Method 1* = reduced all S-S with DTT and alkylated; analyze by SDS-PAGE
      Result: all other selenoproteins but mutated SelL shifted (wt SelL did shift)
   b. *Method 2* = pretreate with *N*-ethylmaleimide, then DTT and alkylation
      Result: no SelL shift observed (Se-Se bond!)

(Supporting Information)

Gladyshev, V. N. *PNAS* **2007**, *104*, 13919.

Identification and Characterization of a Selenoprotein Family Containing a Diselenide Bond

Summary

Isolated first Se-Se containing natural protein, SelL

Demonstrated a single SECIS element can direct the incorporation of 2 Sec

Many questions remain:

- Does a catalytic role exist for Se-Se in proteins?
- Why is SelL only found in aquatic organisms?
- Do other proteins contain this interesting motif?

Gladyshev, V. N. *PNAS* **2007**, *104*, 13919.